Dendritic Cell Vaccines for Brain Tumors

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KEYWORDS

• Brain tumor • Immunotherapy • Dendritic cells
• Cancer vaccines

Dendritic cells (DC) have long been regarded as the most potent antigen-presenting cells within the immune system. Their ability to sample environmental antigens and stimulate T-cell activity in a major histocompatibility complex (MHC)–restricted manner has attracted much attention given the poor antigen-presenting ability and immunogenicity of tumor cells.[1,2] Although DCs constitute approximately 0.3% of all circulating blood leukocytes, they serve as the sentinels of the immune system and are found nearly ubiquitously throughout the body.[3] In their immature state, DCs are highly specialized antigen samplers capable of surveying their microenvironment through several mechanisms including engulfment, macropinocytosis, and receptor-mediated endocytosis.[3] On encountering an antigen the DC processes it through MHC pathways and directs it to the cell surface to form an MHC-peptide complex (**Fig. 1**). In line with traditional antigen presentation following uptake from the environment, many antigens are channeled through MHC-class II pathways with resultant MHC-peptide complexes being capable of stimulating CD4+ T cells. In addition, DC possesses the unique ability to "cross-present" acquired antigens. In this process, DC endosomes release captured antigenic material into the cytosol where it is broken down by proteasomes.[4] The degraded peptides are then transported to the endoplasmic reticulum by a transporter-associated protein and bound to MHC-class I molecules for presentation to CD8+ T cells.[5,6] These distinct mechanisms allow DCs to stimulate T cells in an MHC-class I and II manner, overcoming classical restrictions in antigen processing and presentation[7] and diversifying the resultant immune response.

DCs are capable of handling a vast range of antigenic mediums. The sources of antigen that have been used in DC immunotherapy include exogenous MHC-restricted peptides, acid-eluted tumor peptides, tumor RNA and cDNA, viral vectors, apoptotic tumor cells, tumor cell lysate, and whole tumor cells. Many of these methods have been used with varying degrees of success. A growing sentiment has emerged, however, which argues for the use of a diverse range of antigens that cover both MHC classes rather than constructing specific MHC-matched peptides. The reasoning for this is multifold. First, stimulating T cells with a broad range of antigens reduces the likelihood of an escape phenomenon in which tumor cells lacking the specific antigens of interest avoid immune detection and continue to grow unhindered. Second, it is now well established that the stimulation of both CD4+ and CD8+ T cells is crucial in the activation and maintenance of antitumor immunity.[7–10] By allowing DCs to present and cross-present antigens on MHC-class II and I molecules, respectively, one avoids having to laboriously engineer peptides for each MHC class.[9,11] Finally, the methods used to load the spectrum of antigens for a particular tumor obviate the need of characterizing each individual antigen used. Although the use of unfractionated tumor material containing unknown antigens has long raised the concern of inducing autoimmunity, particularly in the form of experimental allergic encephalomyelitis, no reports of this complication have been seen following DC vaccination in humans to date.[3]

DC vaccine is defined as DCs loaded with antigens (eg, those found on glioma), which are

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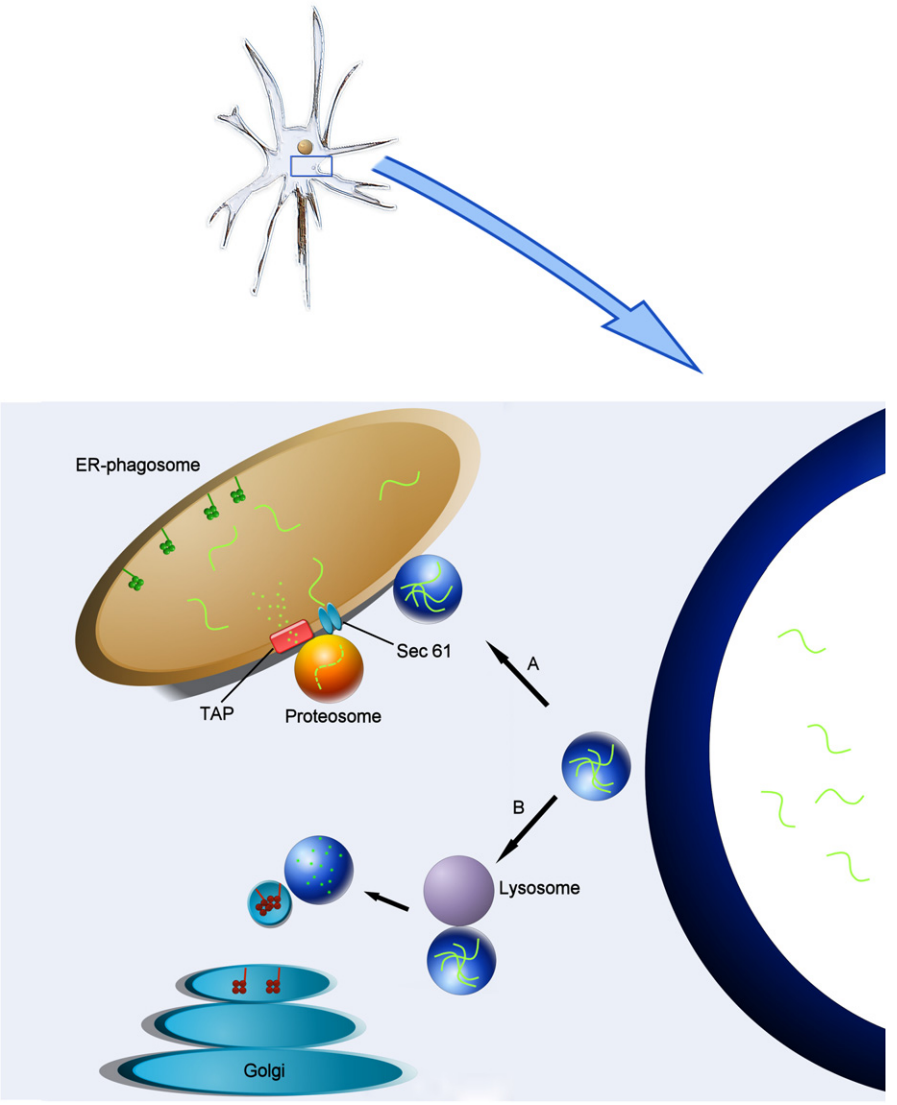

Fig. 1. Schematic of DC antigen processing and presentation by distinct MHC-I and MHC-II targeted pathways. Foreign antigens are sampled from the environment by dendritic cell phagocytosis or pinocytosis. Once vacuolized, antigen-containing vesicles are directed down one of two MHC pathways that result in cell surface presentation. (*A*) Antigen-containing vesicle encounters endoplasmic reticulum (ER)-phagosome. Antigen is retro-translocated into the cytoplasm by Sec 61 where proteosome complexes mediate peptide degradation. The resultant epitopes are translocated back into the ER-phagosome by the transporter-associated protein (TAP), where they are loaded onto MHC-I complexes and extruded for membrane integration and antigen presentation on the cell surface. (*B*) Antigen-containing vesicle encounters lysosome, which cleaves peptides using acid proteases. MHC-II complexes formed within the ER and subsequently processed and extruded from the Golgi apparatus are transported to peptide-containing endosomes for antigen loading.

administered to patients to induce an antigen-specific T-cell mediated antitumor response.[12] Although immature DC are not functionally ideal for the loading of antigens, they are unable to activate lymphocytes until an inflammatory signal or pathogen induces their maturation.[3,9,11] Some groups argue that ex vivo maturation of DCs through CD40L or interferon (IFN)-γ[13] is necessary before vaccine administration to ensure proper antigen presentation and T-cell activation.[14–17] Others maintain that maturation occurs naturally, and that no prior stimulus is required.[18] In the process of maturation, DCs lose their ability to uptake and process antigens. Moreover, they exchange their immature molecular signature for a mature (CD83+) phenotype, increasing

expression of MHC-antigen complexes, lymphocyte costimulatory molecules (eg, CD80/B7-1 and CD86/B7-2), tumor necrosis factor and tumor necrosis factor receptor molecules (eg, CD40), and many chemokines and chemokine receptors (eg, interleukin [IL]-12, -15, -18) to aid in T-cell recruitment and DC navigation to lymphoid tissues (as reviewed by Steinman and Dhodapkar[11] and Soling and Rainov[9]).

On localization to lymph organs rich in naive T cells, mature DCs present their processed antigens in a MHC-restricted manner. Through various interactions they are able to mobilize many different arms of the immune system, including CD8+ cytotoxic T cells (CTLs), CD4+ helper T cells, natural killer (NK) cells, and NK-like T cells.[11] Each of these cell types plays an essential role in the antitumor response (**Fig. 2**). T cells expressing CD8 coreceptors recognize and lyse tumor cells in an MHC-class I restricted fashion, and have received much of the credit as the primary effector cell in immunotherapy. CD4+ T cells have traditionally been known for their part in the expansion and maintenance of CD8+ CTLs, secretion of stimulatory cytokines, and the induction of lasting immunity. Their critical role in immunotherapy has become increasingly appreciated over the past few years, as studies have demonstrated that their absence may result in deficient DC maturation and CTL tolerance.[7,8] Finally, NK and NK-like T cells have a unique niche in the leukocyte armament, being able to

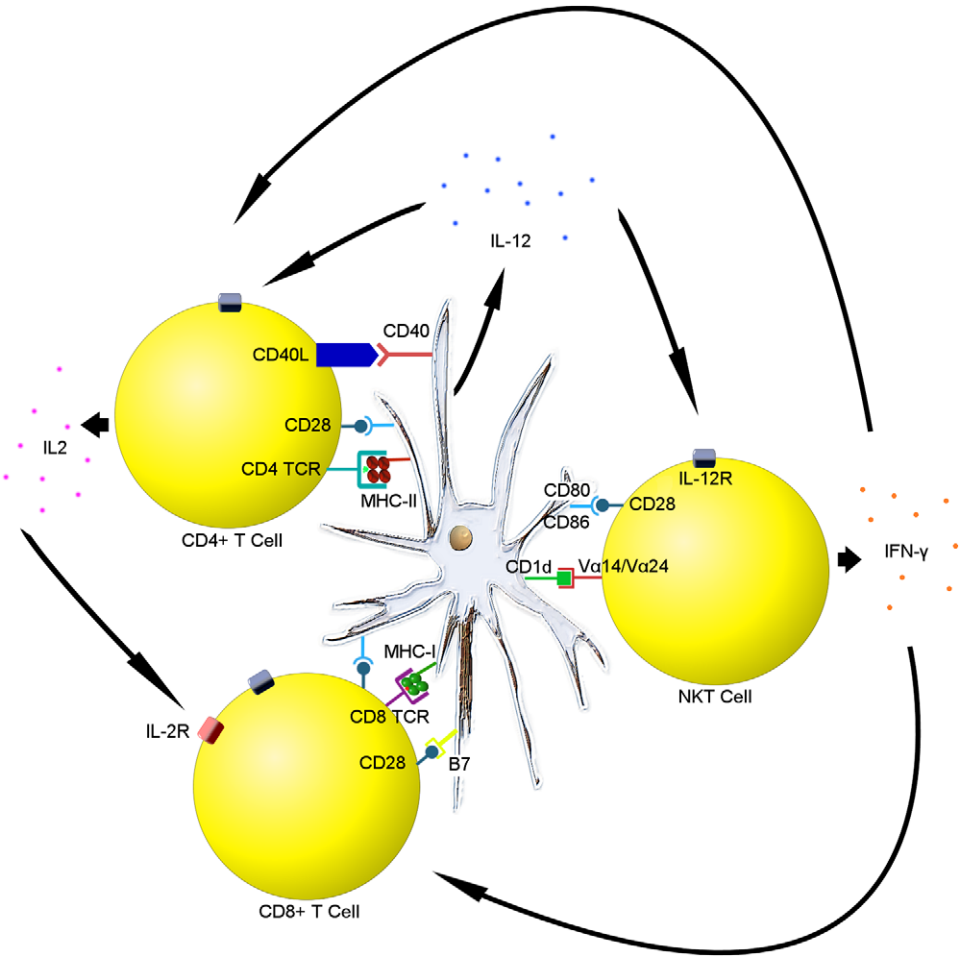

Fig. 2. Diagram depicting the multiple DC-lymphocyte interactions that take place in the immune cascade following antigen processing and presentation by DCs. DC activation of NK T cells through self ligands (not shown) and IL-12 results in IFN-γ release and subsequent activation of CD4+ and CD8+ T cells. CD4 and CD8 T cell receptors (TCR) interact with peptide-MHC II and I complexes, respectively. CD40L-CD40 interactions between CD4+ T cells and a DC and CD28-B7 interactions between CD8+ T cells and a DC are critical costimulatory interactions that must occur for appropriate T-cell signaling and immune responsiveness.

recognize and kill tumor cells that do not express surface markers, such as MHC-class I. Although the exact mechanism of recognition and elimination of tumor cells in the absence of MHC-restriction is yet to be elucidated, they serve as an important complement in the killing of tumor cells that may possess diminished surface marker presentation and avoid CTL detection.[3,11]

ANIMAL MODELS

Preclinical animal models explored many of the methodologies and safety concerns regarding DC vaccines. In the late 1990s, Liau and colleagues were among the first to describe the effectiveness of DC vaccines in the antitumor immunity of gliomas using a rat model.[19] Over the past decade, many groups have published similar studies with varying permutations in the choice of antigen, timing of vaccinations, and measures of therapeutic efficacy. The dissimilarities between study designs make it difficult to compare methodologies and their associated outcomes. Their value, however, lies within their ability to demonstrate the effectiveness of multiple different DC vaccine techniques in inducing antigen-specific cytotoxicity in vitro and in vivo. These studies have shown improved survival outcomes and the safety of the strategy.

Many of the initial animal studies were key in evaluating the effectiveness of DC vaccination techniques for tumors located in the ''immunologically privileged'' central nervous system (CNS). Antigen sources used included synthetic peptides[20,21]; acid-eluted tumor peptides[19]; tumor lysate[21–26]; DC-tumor fusion cells (FCs)[27]; and antigen-containing vectors, such as cDNA-RNA carrying viruses[28,29] and tumor extract carrying liposomes.[30] Many of these studies adopted strategies for antigen loading of DCs from previous experiments in peripheral neoplasms and initial treatment schedules were similarly based on regimens that showed promise in non-CNS immunotherapy.[19] Nevertheless, the timing of DC administration variedly widely, with vaccinations being given before tumor inoculation,[22,24,26–28] simultaneously with,[21,23,25] or some time following tumor implantation.[19,24,27,28,30] The number of vaccinations ranged from two to five times across the various studies.

Overall, most groups concluded that vaccination with antigen-pulsed DC was able to produce a significant antitumor immune response. This was evidenced by increased overall survival in rats and mice; greater degrees of T-cell infiltration (primarily CD8+) on histologic analysis of tumors; and more robust antitumor cytotoxicity assays when splenocytes were incubated with mouse glioma in vitro in immune responders. Although the studies conceded that pretumor vaccination resulted in greater survival in animal models, there are conflicting reports regarding the efficacy of DC vaccines when administered simultaneously or following tumor implantation.[21–25] Groups reporting no improvement in survival with DC vaccination after an established tumor suggest that it may be caused by the immune system failing to generate an appropriate response quickly enough to counteract a rapidly growing tumor within a confined cranium.[21,24] Studies in which T cell–mediated tumor killing was achieved showed, however, that the animals that did respond to DC vaccination obtained lasting antitumor memory, with significantly improved survival following tumor rechallenge compared with unvaccinated controls.[21,22,26]

These animal studies played an important role in alleviating some of the concerns regarding DC immunotherapy for CNS tumors. Experimental allergic encephalomyelitis in particular was perhaps one of the most feared side effects, because previous studies have shown this lethal form of autoimmunity to occur following the injection of glioblastoma tissue into animals.[31] Such signs of autoimmunity were not reported, however, in the more recent studies conducted to date.[21,22] In addition to the information they provided regarding the applicability of different antigen sources and treatment schedules, the preclinical reports corroborated the idea that DC immunotherapy could be effectively used for intracranial neoplasms, and set the stage for further clinical studies.

CLINICAL TRIALS

In 2000, Liau and colleagues published a case report on the first brain tumor patient to be treated with DC-based immunotherapy.[32] A patient with histologically confirmed glioblastoma multiforme (GBM) received three biweekly intradermal injections of DCs pulsed with acid-eluted, allogeneic MHC-I matched GBM peptides. Although the authors were able to appreciate an immune response as evidenced by an increased infiltration of CD3+ T cells in postvaccination tumor, there was no objective clinical response from the treatment. The patient's poor Karnofsky performance score in addition to the possible lack of antigen homology between the allogeneic GBM and the patient's tumor may have contributed to the lack of clinical response or prolonged survival.

In a phase I dose-escalation clinical trial, 12 GBM patients were treated using DCs pulsed

with autologous acid-eluted MHC tumor peptides in a dose-escalation study.[33] Patients were separated into three cohorts, each receiving 1, 5, or 10 $\times$ 10^6 DCs per injection. Subjects tolerated the procedure well with no signs of autoimmunity. There were only minimal grade I toxicities related to the study vaccine, which were distributed similarly across all three dose groups. Although this study was not powered to measure efficacy, patients undergoing DC-based immunotherapy seemed to have an increased median time to progression (TTP) (15.5 months) and overall survival (23.4 months) compared with historical controls. Of note, tumor burden and disease progression at the time of vaccination was a critical determinant of systemic CTL activity, tumor infiltration by T cells, and overall survival. All patients who generated a systemic CTL response showed no MRI evidence of progressive disease at the time of vaccination. Conversely, no patient with actively progressive disease developed statistically significant cytotoxicity. Moreover, only patients possessing minimal tumor burden at the time of vaccination were found to have tumor-infiltrating lymphocytes (TILs) on postvaccination tissue examination. These findings suggest that active tumor progression or bulky residual burden can debilitate the initiation and propagation of an antitumor response. Interestingly, expression of the inhibitory cytokine transforming growth factor-β2 (TGF-β2) was found to be inversely proportional to the number of TILs found in tumor tissue following vaccination (IL-10 was not), implicating TGF-β2 as a possible mediator of immune evasion following vaccination. This study argues for the need for maximal resection or minimal residual disease to improve the efficacy of DC-mediated immunotherapy for glioma. Importantly, this clinical trial established the feasibility, safety, and immunologic potential of DC vaccines for brain tumor patients.

Yu and colleagues[34] reported another phase I clinical trial using DC pulsed with autologous, acid-eluted peptides for glioma patients. In this study, nine patients with newly diagnosed malignant glioma received three biweekly subcutaneous injections of DCs loaded with acid-eluted tumor peptide. Systemic antitumor cytotoxicty was detected in four of the seven patients assessed; intratumoral CD8+ CTL and CD45RO+ memory T-cell infiltration was found in two of the four patients who underwent a second resection because of tumor progression. Patients receiving DC vaccination were found to have an increased median survival (455 days) compared with that of those in the control group (257 days).

Kikuchi and colleagues[35] used DC-glioma FCs to vaccinate glioma patients in their phase I clinical trial. Eight patients with malignant gliomas received FCs intradermally every 3 weeks, with the total number of injections ranging from one to nine. An increased percentage of NK cells were found on FACS analysis in the peripheral blood of patients. In addition, an increase in IFN-γ release in peripheral blood mononuclear cells (PBMC) tumor coincubation with both autologous and allogeneic glioma was seen following DC vaccination. Two patients experienced a minor response and no serious side effects were observed. These findings suggested that nonspecific antitumor cytotoxicity may play a role in the DC-based immunotherapy of glioma.

Kobayashi and colleagues[36] vaccinated five patients with autologous glioma RNA-pulsed DCs. They were able to demonstrate the presence of a strong CD8+ CTL response against autologous glioma accompanied by a weaker NK cell–mediated cytotoxicity in their patients. This finding was significant in three of the five patients treated. Notably, in the two patients with minimal immune responses, a constitutively increased expression of the inhibitory cytokine IL-10 and decreased expression of IFN-γ by CD8+ T cells was found in vitro.

Yamanaka and colleagues[37,38] compared different routes of DC injection in their phase I-II clinical trial of 10 patients. DCs were pulsed with autologous tumor lysate and administered to patients intradermally (N = 5) or both intradermally and intratumorally by an Ommaya (N = 5) reservoir every 3 weeks for a total number of injections ranging from 1 to 10. Immunologically, they observed an increased percentage of NK cells and increased T cell–mediated antitumor activity. In addition, there was an increased intratumoral infiltration of CD4+ and CD8+ T cells in the two patients who underwent reoperation following vaccination. Radiographically, the two minor responses seen were in patients included in the combined intradermal-intratumoral administration group, suggesting that the additional intratumorally injected DCs may stimulate a more efficient antitumor immune response.

Wheeler and colleagues[39] published a report examining the correlation between thymic function, as manifest through CD8+ recent thymic emigrant production, age, and patient outcome in 17 GBM patients undergoing DC immunotherapy. They found that thymic function, as reflected by its ability to produce CD8+ T cells, was directly proportional to good clinical outcomes in mice and human GBM patients and inversely proportional to age. Although patient age has long been

a predictor of mortality and prognosis, their findings suggest that it is actually thymic function, which is inversely correlated with age, which may be the more telling factor. This nonspecific immune parameter may later serve as an important prognosticator in glioma immunotherapy.

Caruso and colleagues[40] conducted a phase I study of nine pediatric brain tumor patients undergoing immunotherapy by autologous tumor RNA pulsed DCs. The cohort was comprised of a wide range of different tumor histologies (**Table 1**). Although they detected a modest increase in antitumor antibodies in some patients, they did not appreciate any increase in T cell–mediated antitumor immunity. This may be explained by their findings that their patients had impaired immunocompetency before the start of the trial. Despite this, they reported clinical responses in three patients during the course of their study.

De Vleeschouwer and colleagues[17] explored the possibility of assessing immunotherapeutic progress through the use of MRI and methionine positron emission tomography. By monitoring contrast-enhancement changes in relation to metabolic uptake ratios they could postulate at which point an immune response had occurred. This group published the findings from their phase I clinical trial of 12 recurrent malignant glioma patients who were vaccinated with tumor lysate-pulsed DC.[16] Interestingly, they were the first to induce DC maturation ex vivo for glioma immunotherapy based on recent evidence arguing that the injection of mature DCs may mediate a more potent antitumor response.[45,46] The extent of resection stressed in this study as prolonged disease-free survival was only achieved in two patients who underwent gross total resection (GTR) before vaccination. Moreover, one patient who received only partial tumor resection suffered grade IV neurotoxicity (National Cancer Institute common toxicity criteria) secondary to vaccination-induced peritumoral edema. As such, they argue that maximal resection may help avoid such dangerous complications during CNS immunotherapy. Akin to the authors' conclusions,[33] this study further champions the need for maximal resection to improve the potential efficacy of vaccination strategies for malignant gliomas.

In a phase I-II study of tumor lysate-loaded DC vaccination for malignant glioma, subcutaneous injections of DCs loaded with tumor lysate were administered biweekly for a total of three injections.[41] Elevated IFN-γ mRNA levels in PBMCs, positive cytotoxicity assays, increased peripheral CD8+ CTLs, and increased infiltration of CD45RO+ memory and CD8+ T cells in progressive tumor corroborated a positive immune response. Additionally, this study reported an increased median survival in patients receiving vaccinations (133 weeks) compared with historical controls (30 weeks), further substantiating the viability of DC immunotherapy for glioma.

After their initial phase I clinical trial, Kikuchi and colleagues[42] continued their work with human patients through a phase I-II series modeled after their animal studies involving DC-glioma FC injection with perivaccination IL-12.[27] Fifteen patients were vaccinated intradermally with FCs on a biweekly basis for a total of three injects per course, with IL-12 administration on days 2 and 5 following each injection. IL-12 was given because it had been shown to enhance the antitumor effects of FCs in mouse models. Similarly, they found that treatment efficacy using FC–IL-12 vaccination in human patients was better than FCs alone. Although, they were able to demonstrate cellular antitumor immunity in only a few of their patients, they observed much improved clinical outcomes, including four partial responses and one mixed response as determined by imaging. Patients tolerated the treatment regimen well and there were no reported signs of autoimmunity despite the use of systemic IL-12.

Walker and colleagues[43] investigated the interaction between chemotherapy and DC vaccines in their phase I clinical trial. Thirteen patients with malignant glioma were treated with six biweekly injections (and every 6 weeks thereafter) of DCs pulsed with autologous irradiated tumor cells. Immunologically, they were able to appreciate an antitumor response by the presence of increased cytotoxic and memory T cells on postvaccination resected tumor. Of the eight patients who received adjuvant chemotherapy in addition to immunotherapy, five were reported to show objective radiologic response to treatment, including one patient who had a complete response. This mirrors findings by Wheeler and colleagues,[47] who through retrospective analysis determined that patients who received chemotherapy following DC immunotherapy did better in terms of overall survival and time to recurrence than patients who received either one alone. Although it was previously believed that chemotherapy and immunotherapy were antagonistic forms of treatment,[48] this and other studies have added to the accumulating evidence that these two therapies may be synergistic in nature.

In a recent paper, De Vleeschouwer and colleagues[14] published an update of their work on DC immunotherapy for brain tumors, including 56 patients with recurrent glioblastoma. Patients received intradermal injections of mature DCs pulsed with autologous tumor lysate according to

three vaccination schedules that varied in regards to frequency of injections and the presence or absence of tumor lysate boosts (see **Table 1**). In addition, delayed-type hypersensitivity (DTH) was assessed in 21 patients from whom enough tumor material could be removed for appropriate testing. The treatment regimens were well-tolerated with the exception of one patient who developed vaccination-induced grade IV neurotoxicity as was mentioned in their previous study[16] and two patients who experienced grade II transient hematotoxicity. Analysis of patient survival and TTP revealed that GTR before vaccination was the only independent predictor of progression-free survival. Younger age (<35) and GTR were predictive of better overall survival, however, only in univariable analyses. Although it did not reach statistical significance, the regimen that included frequent vaccinations with tumor lysate boosting seemed to have improved progression-free survival. Interestingly, DTH reactivity was not shown to have any correlation with clinical outcome.

Recently, Wheeler and colleagues[44] reported on their phase II trial in which they treated 34 patients with new or recurrent glioblastoma. Patients received a total of four subcutaneous injections of autologous tumor lysate-pulsed DCs on weeks 0, 2, 4, and 10. Primary outcomes of interest were TTP and time to survival. Immunologic responses were quantified through measuring the differential expression of IFN-γ mRNA in lysate pulsed DCs expanded from PBMCs collected before and after vaccination. Using normalized IFN-γ production values as previously reported,[49] 17 of the 31 patients tested showed a positive vaccine response ($\geq$1.5-fold expression) after three vaccinations (responders). The magnitude of increased IFN-γ expression correlated logarithmically with time to survival, however, only in vaccine responders. This finding was striking in that it was the first immunologic predictor of immunotherapy outcome to achieve statistical significance, likely because of the large number of vaccine responders in this trial. Clinically, vaccine responders had significantly longer time to survival (642 $\pm$ 61 days) compared with nonresponders (430 $\pm$ 50 days). Moreover, disease-free progression in vaccine responders was improved by 4.5 months, with responders and nonresponders having TTPs of 308 $\pm$ 55 days and 167 $\pm$ 22 days, respectively. It should be noted that these trends were not significant in patients with recurrent glioblastoma, only in those with newly diagnosed tumors. Finally, it was found that patients in this study experienced a 186- to 190-day increase in TTP when the course of DC injections was followed by adjuvant chemotherapy, compared with DC therapy alone. This treatment effect was observed indiscriminately between responders and nonresponders, with differences only appreciable when comparing patients with fivefold IFN-γ increase with all others. These findings supported recent data suggesting that chemotherapy may possibly potentiate the clinical effects of DC-based immunotherapy.[47,48]

CURRENT STATUS OF DC VACCINES FOR BRAIN TUMORS

Safety

DC immunotherapy for brain tumors, throughout the 16 different clinical trials and over 200 patients treated to date, seems to be well tolerated across all variations in treatment protocols. A notable exception was one patient who experienced a grade IV neurotoxicity following DC administration, which was believed to be caused by peritumoral edema from the gross residual tumor.[14] Another patient, interestingly, developed a subcutaneous glioblastoma with single lymph node involvement following DTH testing.[41] Despite these outliers, most groups have predominantly reported grade I and II toxicities in response to DC vaccine administration, with no treatment-associated deaths or permanent neurologic defects. The most common reason for discontinuing DC-immunotherapy was tumor recurrence or progression, as is the case with any other treatment modality for glioblastoma. Overall, the relative lack of serious adverse effects supports the safety of DC-based immunotherapies when used in the management of brain tumor patients.

Measures of Outcome

One of the major criticisms of immunotherapy has been the lack of evidence supporting its objective clinical benefit (by MRI response criteria) despite the numerous studies that have validated its immunologic antitumor response.[50] This assertion was posed while evaluating clinical outcomes of immunotherapy using antiquated imaging criteria, however, which many now argue may not be an appropriate means of assessment in the presence of improved imaging technologies and greater emphasis on disease control and stability, quality of life, and overall survival.[43,51,52] Moreover, although systemic evidence of antitumor responses following DC immunotherapy has been demonstrated on many occasions both in vitro and in vivo, its correlation with actual tumor lysis in human patients is inconsistent at best.

Several groups have tried to determine an immunologic correlate of clinical efficacy in their

Table 1
Summary of phase I and II clinical trials of DC vaccination for CNS tumors

Series	Number of Patients (Type of Trial)	Tumor Characteristics	Antigen Source	Dendritic Cell Characteristics	Immunologic Response	Clinical Response	Toxicity
Liau et al (2000)[32]	1 (case report)	Recurrent GBM (N = 1)	Acid-eluted allogeneic MHC-I matched GBM	PBMCs differentiated with GM-CSF and IL-4; three biweekly i.d. injections	In vitro T-cell proliferative response against allogenic tumor peptides	None	None
Yu et al (2001)[34]	9 (phase I)	AA (N = 2) and GBM (N = 7)	Autologous acid-eluted tumor peptides	PBMCs differentiated with GM-CSF and IL-4; three biweekly s.c. injections	JAM assay: systemic T-cell mediated cytotoxicity against tumor (N = 4 of 7 tested) IHC: increased infiltration of CD8+ and CD45RO+ T cells in tumor following vaccination (N = 2 of 4)	Increased median survival time compared with controls (455 d vs 257 d)	Mild transient fever, nausea and vomiting (N = 1), generalized lymphadenopathy (N = 1)
Kikuchi et al (2001)[35]	8[a] (phase I)	AA (N = 3) and GBM (N = 5)	Autologous DC-tumor fusion cells	PBMCs differentiated with GM-CSF, IL-4, and TNF-α; one to nine injections i.d. every 3 wk	FACS assay: increased percentage of NK cells (N = 4 of 5 tested) ELISA: increased PBMC IFN-γ release with autologous and allogeneic tumor (N = 6 of 6 tested)	Minor response (N = 2) (A) resolution of intractable headache (B) improved hemiparesis	Erythema at injection site (N = 1)
Kobayashi et al (2003)[36]	5	GBM (N = 5)	GFP transfected autologous tumor RNA with cationic lipid	PBMCs differentiated with GM-CSF and IL-4	In vitro cytotoxicity against tumor by CD8+ (major) and NK-like T cells (minor) (significant in N = 3; minimal in N = 2) ELISA: increased IL-10 and decreased IFN-γ production by CD8+ T cells in patients with minimal CD8 cytotoxicity	Not reported	None

Yamanaka et al (2003)[37]	10 (phase I and II)	AA (N = 3) or GBM (N = 7)	Autologous tumor lysate (with KLH)	PBMCs differentiated with GM-CSF and IL-4; one to ten injections i.d. (N = 5) or i.d and i.t. (N = 5), once every 3 wk	FACS assay: increased percentage of NK cells (N = 5 of 5 tested) and CD8-, CD16-, and CD19-positive T cells (N = 4 of 5 tested) DTH: positive reaction to tumor lysate (N = 3 of 6 tested); ELISPOT: increased T-cell mediated antitumor activity (N = 2 of 5 tested) IHC: increased infiltration of CD4+ and CD8+ T cells in patients undergoing reoperation for tumor progression (N = 2 of 2)	Minor response (N = 2) (A) decreased contrast-enhancing portion of lesion (B) improvement in convulsions and decrease in contrast-enhancing portion of lesion	Mild headache (N = 1); erythema at injection site (N = 2)
Wheeler et al (2003)[39]	17 (phase I and II)	New or recurrent GBM	Autologous tumor lysate	PBMCs differentiated with GM-CSF and IL-4; three injections s.c. biweekly (a fourth 6 wk after third in phase II patients)	Not reported; trial conducted to study the relationship between CD8+ recent thymic emigrants and survival		
Caruso et al (2004)[40]	9[a] (phase I)	PA (N = 1), AA (N = 1), GBM (N = 2), medulloblastomas (N = 1), ependymomas (N = 3), and pleomorphic xanthoastrocytomas (N = 1)	Autologous tumor RNA	PBMCs differentiated with GM-CSF and IL-4; zero to five injections i.v. and i.d. biweekly	ELISA: modest increase in specific antitumor antibodies (N = 2 of 5 tested) IFN-γ–producing assays: no significant antitumor response (N = 4 of 4 tested) T-cell proliferation assay: no significant antitumor response (N = 3 of 3 tested)	Tumor free, stable disease at 21, 6, and 2 mo follow-up (N = 3 of 7 treated)	None

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Table 1
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Series	Number of Patients (Type of Trial)	Tumor Characteristics	Antigen Source	Dendritic Cell Characteristics	Immunologic Response	Clinical Response	Toxicity
de Vleeschouwer et al (2004)[17]	1[a] (case report)	Postradiation AA (N = 1)	Autologous tumor lysate	PBMCs differentiated with GM-CSF and IL-4; DCs matured with TNF-α, IL-1β, and PGE_2; six injections i.d. first two every 2 wk, then every mo thereafter	DTH: positive reaction to tumor lysate (N =1) MRI: transient contrast enhancement after fifth vaccine MET-PET: transient increased metabolic uptake around resection cavity	Tumor-free survival (60 mo following first vaccination)	None
Yu et al (2004)[41]	14 (phase I and II)	Recurrent AA (N = 3) or GBM (N = 9) and new AA (N = 1) or GBM (N = 1)	Autologous tumor lysate	PBMCs differentiated with CM-CSF and IL-4; three injections s.c., biweekly	qPCR: IFN-γ mRNA accumulation in PBMC (N = 6 of 10 tested) JAM assay: systemic T cell mediated antitumor cytotoxicity (N = 1 of 1 tested) Tetramer staining: increase in CD8+ antigen-specific T-cell clones (N = 4 of 9 tested) IHC: CD45RO+ memory T cells and CD8+ CTLs in resected progressive tumor (N = 3 of 6)	Increased median survival compared with controls (133 vs 30 wk, respectively)	Transient headache (N = 3), erythema at the injection site (N = 1), generalized seizures (N = 2)
Rutkowski et al (2004)[16]	12[a] (phase I)	AA (N = 4) and GBM (N = 8)	Autologous tumor lysate	PBMCs differentiated with GM-CSF and IL-4; DCs matured with TNF-α, IL-1β, and PGE_2; two to seven injections i.d., first two separated by 2 wk, then monthly thereafter	DTH: positive response to tumor lysate (N = 6 of 8 tested)	Following STR: stable disease (N = 1 of 6), partial response (N = 1 of 6) Following GTR: continuous complete remission 5 y after DC vaccine (N = 2 of 6)	Reversible grade IV neurologic deficits and lethargy (N = 1), grade II hematotoxicity (N = 2), transiently increased morning stiffness (N = 1), night sweats (N = 1), meningismus (N = 1)

Kikuchi et al (2004)[42]	15 (phase I and II)	AA (N = 9) and GBM (N = 6)	Autologous DC-tumor fusion cells	PBMCs differentiated with GM-CSF, IL-4, and TNF-α; three to nine injections i.d., once every 2 wk; systemic IL-12 given 2 and 5 d after each FC injection	FACS analysis: percentage of cell types did not change significantly (N = 7 of 7 tested) ^{51}Cr release assay: increased cytotoxic activity (N = 2 of 8) Intracellular ELISA (CD8+ T cells): increased IFN-γ (N = 1 of 7) IHC: Robust infiltration CD8+ CTLs in patients undergoing reoperation for tumor progression (N = 2)	MRI/CT: Partial response (N = 4 of 15); mixed response (N = 1 of 15); stable disease (N = 2 of 15)	Transient grade I fever (N = 4), generalized convulsions (N = 1), erythema at injection site (N = 13), transient liver dysfunction (N = 6), leukocytopenia (N = 7)
Yamanaka et al (2005)[15]	24 (phase I and II)	Recurrent AA (N = 6) and GBM (N = 18)	Autologous tumor lysate ($\pm$KLH)	PBMCs differentiated with GM-CSF and IL-4; $\pm$OK-432 for DC maturation (phase II); 1–22 injections i.d. or i.t. (by Ommaya) every 3 wk; immature DCs (phase I) and both immature and mature DCs (phase II)	DTH: Positive response to tumor lysate (N = 8 of 17 tested) ELISPOT assay: tumor-specific CTLs increased (N = 7 of 16)	MRI/CT: partial response (N = 1); minor response (N = 3); no change (N = 10); significantly increased median survival (480 vs 400 d)	Mild headache (N = 1); mild erythema at cervical injection site (N = 7)

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Table 1
(*continued*)

Series	Number of Patients (Type of Trial)	Tumor Characteristics	Antigen Source	Dendritic Cell Characteristics	Immunologic Response	Clinical Response	Toxicity
Liau et al (2005)[33]	12 (phase I)	New (N = 7) or recurrent (N = 5) GBM	Acid-eluted autologous tumor peptides	PBMCs differentiated with GM-CSF and IL-4; 3 injections i.d., once every 2–4 wk; dose escalation	Alamar blue CTL assay: systemic tumor specific CTL (N = 6 of 6 tested) IHC: CD8+/CD45RO+ memory T-cell infiltration in tumors of patients undergoing reoperation for progression who subsequently had >30 mo survival (N = 4 of 4); no increased TILs in tumors of patients undergoing reoperation who subsequently had <30 mo survival RT-PCR: lower expression of TGFβ_2 in tumor of patients with detectable TILs	Partial response (N = 1); increased TTP (18.3 mo vs 8.2 mo in controls) and median survival (correlated with CTL response and minimal tumor burden)	Fever or flu-like symptoms (N = 4), nausea and vomiting (N = 3), erythema at injection site (N = 2), LAD (N = 2), fatigue (N = 5), seizures (N = 1)
Walker et al (2008)[43]	13 (phase I)	AA (N = 4) and GBM (N = 9)	Irradiated tumor cells	PBMCs differentiated with GM-CSF and IL-4; 2–13 injections i.d., first six biweekly, thereafter every 6 wk	IHC: increased CD8+ and CD45RO+ T-cell infiltration in tumors of patients undergoing reoperation (N = 3 of 3)	Response to adjuvant chemotherapy: complete (N = 1 of 8 treated), partial (N = 4 of 8 treated), minimal/none (N = 3 of 8 treated)	None

de Vleeschouwer et al (2008)[14]	56[a]	Recurrent GBM	Autologous tumor cell lysate	PBMCs differentiated with GM-CSF and IL-4; DCs matured with TNF-α, IL-1β, and PGE_2; three to nine i.d. injections (A) wk 1 and 3 then every 4 wk thereafter (B) first five every 2 wk, then every 4 wk (C) four weekly; boosts with autologous tumor lysate	DTH: positive reaction to tumor lysate (N = 9 of 21 tested) before vaccination, with no correlation with survival	Significantly improved PFS in adults of cohort C; significantly improved overall survival in patients <35 y old and PFS in patients with GTR	Grade IV neurotoxicity (stupor) (N = 1), grade II hematotoxicity (N = 2), transient increase in focal neurologic signs (N = 6), headache (N = 9), vomiting (N = 2), flu-like symptoms (N = 3), increased frequency of seizures (N = 4), fatigue (N = 7), myalgias (N = 3), erythema around injection site (N = 56)
Wheeler et al (2008)[44]	34 (phase II)	New (N = 11) or recurrent (N = 23) GBM	Autologous tumor lysate	PBMCs differentiated with GM-CSF and IL-4; 4 injections (three biweekly, then fourth 6 wk after third) s.c.	qPCR: progressively increased IFN-γ after vaccinations, peaking after 3 vaccinations (significantly in 17 of 31 tested)	TTS and TTP significantly longer (but not in recurrent patients alone); TTS correlated logarithmically with postvaccine IFN-γ response magnitudes exclusively in responders; significant increase in TTP during postvaccine chemotherapy interval compared with TTP following vaccine alone in both responders and nonresponders (N = 19)	Cutaneous GBM with single lymph node involvement at site of DTH testing

Abbreviations: AA, anaplastic astrocytoma; CT, computerized tomography; CTLs, cytotoxic lymphocyte; DTH, delayed-type hypersensitivity; GBM, glioblastoma multiforme; GFP, green fluorescent protein; GM-CSF, granulocyte-macrophage colony–stimulating factor; GTR, gross total resection; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; KLH, keyhole limpet hemocyanin; MRI, magnetic resonance imaging; PA, pilocytic astrocytoma; PBMCs, peripheral blood mononuclear cells; PFS, progression-free survival; PGE, prostaglandin E; TNF, tumor necrosis factor; TTP, time to progression; TTS, time to survival.

[a] Study includes pediatric patients.

phase I-II studies, including such measures as DTH,[14–16] the presence of TILs,[33,34,41–43] and antitumor immunity in vitro from systemic CTLs.[15,32–34,36,37,41] Results have been mixed, particularly with DTH, which was only shown to be predictive of improved survival in one study to date.[15] TILs in relapsed tumors and systemic antigen-specific CD8+ antitumor T cells following vaccination are ubiquitously found in patients who seem to respond to immunotherapy; however, they are not prognostically predictive because many nonresponders also present with such cells. It is thought that the microenvironment of the tumor itself, correlated with immunosuppressive cytokine release (eg, TGF-β), may inhibit the exacting of actual tumor killing despite sufficient cellular immunity.[33,36] Questions regarding which biologic indices are predictive of clinical outcome continue to be elucidated as larger cohorts are investigated in multicenter phase II clinical trials for glioblastomas (eg, DCVax). Presently, TTP and overall survival remain the best measures of efficacy in DC immunotherapy.

Methods of DC Vaccine Development and Administration

Notwithstanding a decade of use, there still remains a great degree of variability in the development and administration of DC vaccines. Only a few studies have systematically examined these differences, resulting in a lack of data regarding the most effective means through which to carry out DC-based immunotherapy for CNS neoplasms. Some of these specifics have been resolved on account of information obtained from animal models or through empiric evidence gleaned from common practice. For example, although there are several different methods through which DC may be acquired, in all of the clinical trials involving DC immunotherapy for glioma to date, they were exclusively manufactured through the differentiation of PBMCs ex vivo. There now exists many methods through which DCs can be produced efficiently and in large enough quantities for clinical trials.[53–56]

Similarly, no studies exist comparing the efficacy of different sources of antigens in propagating antitumor immunity in human patients. The vast repertoire of antigen-loading strategies includes whole tumor cells,[27,35,42] apoptotic tumor cells,[43] acid-eluted tumor peptides,[19,32–34] synthetic peptides,[20,21] tumor lysate,[14–17,21–26,38,39,41,44] and tumor cDNA-RNA.[28,36,40,57] The effectiveness of these methods in stimulating DC-mediated antitumor immunity has primarily been studied in animal models for proof-of-principle rather than comparative analyses. Although some animal studies have evaluated the efficacy of different sources of antigens in stimulating a DC-mediated antitumor response,[21,28] the choice of antigenic stimuli in clinical trials seems largely based on previous work with preclinical models and theoretical considerations. Clearly, prior experience with a particular DC vaccination protocol allows for ready transition from bench to bedside. Theoretically, however, methods using a wide range of autologous tumor antigens have been favored over peptide selection. This allows the vaccine to target all tumor-associated antigens without requisite characterization, helping avoid clonal selection of antigen-loss variants and subsequent tumor-escape.[58] Choice of antigen must also be considered for pragmatic reasons, because poor availability of resected tumor tissue may favor the use of cDNA-RNA to pulse DCs because these antigens are readily amplified through molecular techniques.[9] Because most of the antigen sources available to immunotherapy have been shown to prime DC appropriately, however, their use may remain largely an empiric choice until future studies comparatively examining their functionality and practicality are conducted.

It is well accepted that antigen loading is most effective when pulsing phenotypically immature DC. The maturation state in which to administer DCs to patients following this step, however, remains unclear. Numerous studies have shown that DC maturation is necessary for effective DC migration[59] and T-cell stimulation,[46,60] making them more effective in generating an antitumor response.[15] Given the need for an inflammatory stimulus or cytokine to induce DC maturation, DCs have been matured ex vivo to theoretically ensure proper functioning once they reach the lymph nodes of the host.[14–17] This is supported by work by Yamanaka and colleagues[15] who found that patients receiving mature DCs experienced a greater overall survival than patients receiving immature ones. Barratt-Boyes and colleagues,[18] however, were able to demonstrate that immature antigen-pulsed DC undergo natural maturation when injected intradermally and are quite capable of stimulating appropriate antitumor T-cell pathways in vivo. Moreover, they argue that the administration of immature DCs may even be superior to that of mature DCs, because the latter have relatively decreased emigration rates from the injection site. Because clinical trials have demonstrated clinical benefit with DC regimens using both immature and mature DC, future studies comparing the two preparations are needed to further evaluate the effect maturation

status has on clinical efficacy and patient survival.[61]

The frequency of DC injections in clinical trials was initially modeled after administration schedules found to be effective in immunotherapy for non-CNS tumors.[19] Since then, most studies have roughly followed a biweekly injection regimen, with number of vaccinations varying from 1 to 22 times (see **Table 1**). Given the many differences in other aspects of the vaccination protocol, it is difficult to compare the efficacy of DC administration frequency between published studies. It has been argued that vaccination should be given expediently following maximal surgical cytoreduction, chemotherapy, or radiotherapy to fully benefit from the rebound in immune function following GTR before tumor recurrence.[52] Although early initiation of DC immunotherapy is encouraged, data from animal[26] and patient[14] studies suggest early follow-up vaccinations are not as critical and may hinder the immune response by causing activation-induced death of recently activated T cells. Instead, these studies have demonstrated that booster injections with tumor lysate alone may be more beneficial in stimulating an antitumor response. Interestingly, many studies have used the testing of DTH using tumor lysate,[14–17,37,44] which may have inadvertently served as a form of booster and improved antitumor immunity. Given the lack of controlled studies addressing these issues, the timing and frequency of DC vaccine administration remains largely based on empiric experience, and requires future studies to determine an optimal schedule.

The optimal dose of DC has similarly been questioned. Even from early preclinical studies, it was evident that low inoculations of DCs could stimulate an antitumor response.[16] Dose escalation protocols in clinical trials have substantiated the finding that DC-mediated immunity is an ''on-off'' rather than a dose-response phenomenon, because increasing numbers of these antigen-presenting cells do not affect the magnitude of the CTL response.[14,33] This is reassuring, because large quantities of autologous tumor lysate-pulsed DC were sometimes difficult to obtain during dose escalation protocols.[33,40]

Finally, the route of DC administration best for immunotherapy is still under investigation. DCs can be administered through a variety of ways, including subcutaneous, intradermal, intralymphatic, intranodal, and intratumoral injections. Several studies in mice and nonhuman primates have examined the differences in lymph node accumulation and T-cell stimulation with each route. Radioisotope tracing studies have shown that intravenous DC administration results in the accumulation of DC within the spleen and liver. This method results in the greatest humoral antitumor response, however, as indicated by increased tumor antigen-specific antibodies.[62–65] Conversely, intradermal,[62,65] intralymphatic,[62] intracranial,[66] intranodal,[59] and subcutaneous[63] injections of DCs have been shown to drain to lymph nodes and induce greater T cell–mediated immunity against tumor antigens compared with intravenous injections in preclinical models. Much attention has been given to the intranodal or perinodal administration of DCs, because lymph nodes are acknowledged as the processing centers responsible in mediating antigen presentation and T-cell activation.[67] Some investigators have questioned how the placement of these injections may alter the potency of the immune response. Recently, Calzascia and colleagues[68] were able to show that the distance from the cervical nodes was not as critical as the location of the tumor itself. Although there was some improved tissue tropism for the CNS when DCs were administered into cervical lymph nodes, they found that the ultimate determinant of homing signals was the residence of the actual tumor, as was evidenced by CNS-tropic T cells following inguinal node DC injection in an intracranial tumor model. To date, only one clinical trial has investigated the differences in patient outcome between two injection routes. Yamanaka and colleagues[15] found that patients who received both intratumoral and intradermal DCs had prolonged survival compared with those who received intradermal DCs alone. Further studies comparing injection sites and modalities in inducing antitumor immunity are still needed.

Patient Selection for DC Vaccines

The increasing volume of studies reporting on the clinical response to DC-based immunotherapy has allowed for the analysis of patient demographics to better determine who may benefit the most from this novel treatment modality. As with traditional therapies for malignant brain tumors, younger patients (<40 years old) receiving DC vaccines seemed to do better in terms of overall survival compared with older patients with similar tumor histologies.[44,69] Although this may in part be attributable to the general trend for younger glioma patients to have better prognoses, one particular study was able to demonstrate that this was primarily because of associated declines in thymus function with increasing age.[39] They maintained that CD8+ T-cell production from the thymus was a prognostic indicator of response

to DC immunotherapy independent and superseding that of patient age.

Another critical patient characteristic that seems to have an effect on clinical outcome is surgical management. Those patients who underwent GTR of their brain tumor experienced significantly better progression-free survival compared with otherwise similar patients with appreciable residual tumor.[14] Moreover, bulky residual tumor or active tumor recurrence at the time of vaccination seems to debilitate the antitumor CTL response.[33]

Finally, patients with newly diagnosed malignant glioma seem to achieve greater response rates than those with recurrent tumors.[44] Although these findings remain to be validated by future studies, it seems that younger patients with newly diagnosed malignant glioma that are amenable to GTR stand to benefit the most from DC-based vaccines.

Synergy of DC Vaccines with Other Therapies

Although much progress has been made in DC-based immunotherapy for CNS tumors, objective clinical responses for vaccinated brain tumor patients remains inconsistent. Consequently, some groups have examined the use of adjuvant treatments to augment the effects of DC vaccination. These methods include adjuvant chemotherapy, cytokine administration, and toll-like receptor agonists.

The use of cytokines to supplement DC-based immunotherapy in human patients is an extension of work done in preclinical animal studies.[24,27,29] Although systemic cytokine administration has only been used in one DC vaccine clinical trial to date,[42] studies conducted in vitro and with human patients have shown that cytokines, such as IL-10,[70] IL-18,[71] and IL-23,[72] may enhance the immune response of effector cells in DC immunotherapy. Because the data regarding this adjuvant modality are scarce, further studies are needed before routine clinical use of systemic cytokines can be considered.

The use of standard treatments, such as chemotherapy to aid immunotherapy, has also been considered. Chemotherapy has traditionally been regarded as an antagonist to the treatment effects of immunotherapy, because of its effects of bone marrow suppression causing lymphopenia. There has also been a belief that the dead apoptotic tumor cells produce immune tolerance, exacerbating the lymphopenic state that results. Mounting evidence argues that these apoptotic tumor cells may provide a rich antigen source for DC and that prompt DC vaccination following chemotherapy may actually provide greater benefit than delaying treatment.[48] Recently, studies have shown that when chemotherapy is used adjuvantly with DC-based immunotherapy, patients experience prolonged overall survival and increased time to disease progression.[43,44,47] Because evidence suggests that chemotherapy in the setting of DC immunotherapy may actually be beneficial rather than obstructive, it may be prudent to further investigate multimodality treatment strategies for the simultaneous treatment of brain tumor patients.

SUMMARY

Over the past decade, DC-based immunotherapy for CNS tumors has progressed from preclinical rodent models and safety assessments to phase I-II clinical trials in over 200 patients, which have produced measurable immunologic responses and some prolonged survival rates. Many questions regarding the methods and molecular mechanisms behind this new treatment option remain unanswered. Results from currently ongoing and future studies will help to elucidate which DC preparations, treatment protocols, and adjuvant therapeutic regimens optimize the efficacy of DC vaccination. Additionally, it is important to characterize the pathways underlying the immunosuppressive microenvironment of brain tumors that currently hinder antitumor responses. Combined with further advances in the manipulation of various lymphocyte subsets, such as regulatory T cells and NK-like T cells, in addition to the usual armament of CD4+ and CD8+ T cells, understanding these immunologic intricacies will help maximize the cellular efficiency of immunotherapeutic techniques. As clinical studies continue to report results on DC-mediated immunotherapy, it will be critical to continue refining treatment methods and developing new ways to augment this promising form of glioma treatment.

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